Surface Expression of a T Cell Receptor β (TCR-β) Chain in the Absence of TCR-α, -δ, and -γ Proteins

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Summary

The antigen receptor expressed by mature T cells has been described as a disulfide-linked α/β or γ/δ heterodimer noncovalently associated with CD3, a complex of transmembrane proteins that communicates signals from the T cell receptor (TCR) to the cell interior. Studies suggest that all component chains must assemble intracellularly before surface expression can be achieved. We described, however, a CD4$^+$/CD8$^+$ transformed murine thymocyte, KKF, that expresses surface TCR-β chains in the absence of γ, δ, and α proteins; these β chains are only weakly associated with CD3-ϵ and CD3-ζ. Furthermore, KKF responds differently to stimulation through TCR-β and CD3-ϵ, a functional dissociation that has been ascribed to a CD4$^+$/CD8$^+$ subpopulation of normal thymocytes. KKF's unique TCR structure may offer an explanation for the functional anomalies observed.

The TCR found on the surface of the majority of murine peripheral T cells is a disulfide-linked α/β heterodimer (1) coexpressed with a complex of transmembrane proteins (γ, δ, ϵ, ζ, and η) collectively called CD3 (2). For any of the components to reach the surface of the cell, they must assemble intracellularly to form a complete TCR-α/β, CD3 $\gamma/\delta/\epsilon$ complex; ζ, though not formally required for surface expression, is needed to achieve normal expression levels (3). Incomplete complexes are rapidly degraded in subcellular compartments (4–6).

While the precise structural, spatial, and stoichiometric relationships among the chains are unknown, there have been reports in human cells of specific associations between TCR-β and CD3-γ (7), TCR-α and CD3-δ (8), and TCR-α and CD3-ζ chains (9). There have also been three reports of novel TCR chain combinations. A human leukemia cell line was shown by Hochstenbach and Benner (10) to express a functional TCR-β/δ heterodimer; this unusual pairing may be explained by the structural and sequence similarily between α and δ. More surprisingly, Goverman et al. (11) demonstrated that a transfected β chain could be expressed on the surface of a mouse T cell lymphoma as a disulfide-linked β-β dimer. Because this transfected gene was chimeric for the constant region of TCR-β and the rearranged variable region of an Ig, the physiological implications of this finding are not clear. Finally, evidence has been described for the expression of TCR-β in the absence of the α chain within normal CD4$^-$/CD8$^-$ and CD4$^+$/CD8$^+$ thymocyte subpopulations from mice transgenic for a rearranged TCR-β chain (12, 13).

The structure of the receptor expressed by immature T cells has not been thoroughly defined, although studies imply that it differs from that of mature T cells. Approximately half of the CD4$^+$/CD8$^+$ thymocytes express the TCR/CD3 complex, albeit at lower levels than that observed on mature cells (14). Maturation of these cells involves two phenotypic changes: a shift from the double-positive (DP)[1] to the single-positive (SP) phenotype and the increase in TCR expression to levels comparable to that of peripheral T cells (reviewed in references 15 and 16). Thymocytes that express low levels of TCR and, by inference, bear the DP phenotype do not have the functional capabilities of their mature counterparts; stimulation of the CD3 complex results in a release of intracellular Ca^{2+}, but will not induce the release of lymphokines (17, 18). Furthermore, Finkel et al. (19, 20) have shown that a proportion of those cells that respond to stimulation through CD3-ϵ respond only minimally to stimulation through TCR-β. This functional dissociation between the TCR and CD3 proteins may reflect a structural dissociation of these components.

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[1] *Abbreviations used in this paper:* DP, double positive; pAS, protein A-Sepharose; SP, single positive.

We have identified and characterized a transformed thymocyte cell line, KKF, that shares both the functional and surface phenotype of the subpopulation of DP cells identified by Finkel et al. (19, 20). The structure of the TCR/CD3 complex expressed on the surface of these cells is unusual; while KKF expresses TCR-β, it does not express surface TCR-α, -γ, or -δ proteins.

Materials and Methods

Cell Lines. KKF, KKB, and KgV were isolated independently from BALB/k mice infected with Gross leukemia virus. The isolation and characterization of KKB and KgV has been described previously (21). These cells are maintained in RPMI, 10% FCS, 10 mM Hepes, 1 mM L-glutamine, and 50 μM 2-ME at 37°C in 5% CO_2. DN1.1 (22) and 2B4 (23) are TCR-γ/δ^- and TCR-α/β^- expressing T cell hybridomas, respectively.

Antibodies. H57-597 (24) and 145-2C11 (25) are monoclonal hamster antibodies specific for mouse TCR-β constant region and mouse CD3-ϵ, respectively. Culture supernatants were used for immunofluorescence analysis (see below), and antibodies purified after a 50% saturated ammonium sulfate cut and absorption with QAE sepharose were used for immunoprecipitation (see below). The monoclonal anti-human CD3-ϵ antibody, 1352, is crossreactive with mouse CD3-ϵ (Kubo, R., unpublished results). The monoclonal anti-TCR-α reagent, H28-760 (26), the polyclonal anti-ζ reagent, H146 (a hamster antiserum raised against the COOH-terminal peptide of the mouse ζ protein), and the monoclonal anti-ζ (anti-ζ-COOH-terminal peptide) antibody, H146-968, were all developed in R. Kubo's laboratory. 3A10 is a monoclonal hamster anti-mouse antibody specific for the Cδ region (27). PE-conjugated GK1.5 and FITC-conjugated Lyt-2 were obtained from Becton Dickinson & Co. (Mountain View, CA) and used for two-color immunofluorescence studies. Culture supernatant from MR 12-4 (mouse anti-Vβ13) and MR 10-2 (mouse anti-Vβ9) were the generous gifts of Osami Kanagawa (Washington University) (28).

Immunofluorescence. KKF cells (5 × 10^5) were distributed in a microtiter plate and incubated for 30 min at 4°C with 100 μl of undiluted hybridoma culture supernatant (in the case of MR 12-4 and MR 10-2) or 30 μl staining medium (PBS, 0.5% BSA, 0.05% NaN_3) with saturating concentrations of 2C11, H57-597, PE-GK1.5, or FITC-Lyt-2. Cells were washed two to four times in 150 μl staining medium and incubated for 30 min at 4°C with the appropriate fluoresceinated secondary antibody (fluoresceinated goat anti-hamster antibody) (Organon Teknika, Malvern, PA) was added to KKF incubated with 2C11 and H57-597, and fluoresceinated goat anti-mouse Ig (Organon Teknika) was added to KKF incubated with MR 12-4 and MR 10-2. Cells were washed two to three times and fixed overnight in a solution of staining medium containing 2% formaldehyde before analysis on a FACS IV® (Becton Dickinson & Co.).

DNA and RNA Isolation and Hybridization. Cellular DNA and RNA from KKF, KKB, and KgV were extracted after cell lysis with guanidine thiocyanate and isolation over a cesium chloride gradient (29). Northern and Southern blots were prepared as previously described (30, 31) and nitrocellulose filters were hybridized to probes specific for Cα, Cβ, Cγ (1-3), Cγ4, the 5'Dβ region, Jβ1, Dβ1 (21), Cδ (isolated in the Hashimoto Laboratory), and the 5'Dδ region (32). Sequencing of the TCR-δ transcript was performed by the anchoring PCR method originally described by Loh et al. (33).

Immunoprecipitation. Cells (2.5-5 × 10^7) were incubated for 30 min at 4°C in PBS containing 10 mM iodoacetamide, then washed two times with PBS before lactoperoxidase/hydrogen peroxide (Figs. 6 and 8 *a*) or IODO-GEN (Pierce Chemical Co., Rockford, IL) (Fig. 7) surface labeling with ^{125}I. The cells were washed in PBS and lysed for 30 min at 4°C in 1% digitonin containing 150 mM NaCl, 10 mM iodoacetamide, 1 mM PMSF, 20 mM triethanolamine, pH 7.8. Cell debris was pelleted after a 2-min, 12,000-rpm spin and the supernatant was precleared overnight at 4°C with immune rabbit serum and protein A-Sepharose (pAS) beads. Another preclear was performed on the supernatant with pAS beads only. Lysates used for precipitations shown in Fig. 7 were not precleared. The sample was finally divided and immunoprecipitated for 1 h at 4°C with the pAS beads precoated with the indicated antibody (beads were washed and swollen in 50 mM Tris, pH 8.5/150 mM NaCl, incubated overnight at 4°C with antibody, which had been preequilibrated in this buffer, and washed before use). The supernatant was discarded and the immunoprecipitates washed five times with the lysis bffer before boiling in unreduced or reduced sample buffer (10% glycerol, 62.5 mM Tris, pH 6.8, 2% SDS, 0.001% bromophenol blue with or without 5% β_2-mercaptoethanol) for 2 min. Electrophoresis was performed as described by Laemmli (34) with a 4% stacking and a 10 or 12% running SDS-PAGE discontinuous gel. For two-dimensional electrophoresis, nonreduced samples were run first on a mini-gel apparatus (Bio-Rad Laboratories, Rockville Centre, NY) through 10 or 12% gels of 0.75-mm width. The lanes were cut out, reduced by incubating in sample buffer with β-mercaptoethanol for 30 min, and placed horizontally onto the stacking gel portion of a 10 or 12% gel of 1.0-mm width. Agarose (0.5%) in running buffer (25 mM Tris, 192 mM glycine, 0.1% SDS, pH 8.3) was applied to seal the gel strip and the gel was run as usual.

Immunoblotting. Digitonin lystates from 2 × 10^8 cell equivalents were precipitated with the indicated antibodies, solubilized in Laemmli sample buffer containing 10 mM iodoacetamide, and subjected to electrophoresis under nonreducing conditions on a 10% gel. The proteins were transferred to nitrocellulose and probed with antibodies as described (35).

Ca^{2+} Measurements. Ca^{2+} assays were performed as described by Finkel et al. (19).

Results

KKF Has the Surface Phenotype of an Immature T Cell. The KKF cell line was isolated from a Gross virus-infected BALB/k mouse and characterized by immunofluorescence for surface staining of antibodies specific for a variety of T cell markers. KKF cells expressed Thy-1, J11d, CD3 (Fig. 1 *a*), TCR-β (Fig. 1 *b*), and were heterogeneous for CD4 and CD8 expression: while the majority of cells were CD4$^+$/CD8$^+$ (DP), a small percentage were CD4$^+$/CD8$^-$ (SP) (Fig. 1 *d*). These subpopulations were subsequently found to be phenotypic variants of the same clone (data not shown). The following results are derived from studies using subclones of KKF that predominantly express the DP phenotype.

KKF Has Not Rearranged its TCR-α Loci. An unusual pattern of TCR gene expression was noted during attempts to determine the clonal relationship of the DP and SP subpopulations. While Northern blot analysis (Fig. 2) revealed β transcripts (1.3 and 1.0 kb) and a δ transcript (1.5 kb), no TCR-α

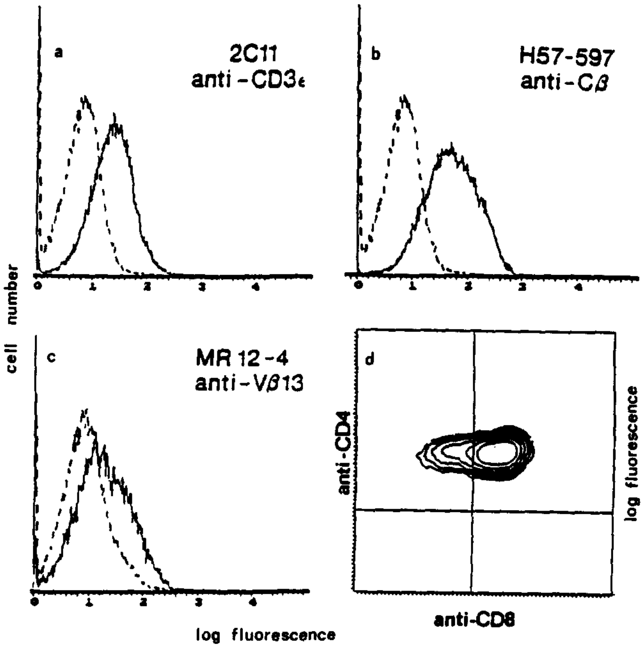

Figure 1. Surface phenotype of KKF. Cells were stained as described in Materials and Methods. (*a–c*) Results of one-color immunofluorescence analysis (fluorescence intensity on the *x*-axis and cell number on the *y*-axis). Negative controls (cells incubated with secondary antibody only) are indicated by the dotted lines, and data from cells stained with (*a*) 145-2C11 (anti-CD3-ε), (*b*) H57-597 (anti-TCR-β), and (*c*) MR-12-4 (anti-Vβ13) are indicated by the solid lines. Isotype-matched antibodies specific for Vβ9 (MR 10-2) did not bind KKF (data not shown). (*d*) Results of two-color analysis of KKF stained with PE-conjugated GK1.5 (anti-CD4) and FITC-conjugated 53-6.7 (anti-CD8).

or -γ gene expression was detected. Because it was possible that the α transcript was present at a level too low in KKF to be detected by Northern analysis, the α and δ loci were examined for gene rearrangements. The entire TCR-δ locus lies between the variable and J regions of the α chain and is necessarily deleted by a functional α rearrangement (36). Therefore, the presence of δ elements implies the absence of α rearrangement.

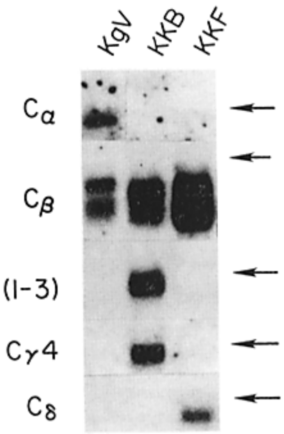

Figure 2. RNA expression of TCR genes. Northern blot analysis was performed on RNA isolated from KKF and two other control Gross virus–transformed cell lines, KKB and KgV. Probes specific for the constant regions of TCR-α, -β, -γ (1-3), -γ-4 (which does not crosshybridize to the other C-γ regions [48]), and -δ regions were hybridized successively to the same blot. Prolonged exposures of the blots probed with Cα and the Cγs did not reveal any hybridization to KKF RNA. The arrows mark the position of the 18S rRNA band (∼1.9 kb in size).

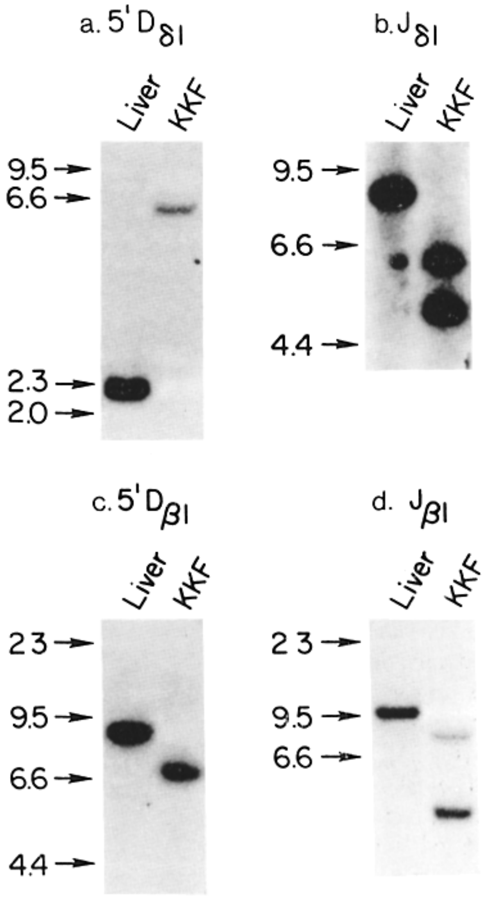

Figure 3. KKF TCR-δ and -β gene rearrangements. EcoR1 digests of BALB/c liver and KKF cellular DNA were hybridized with probes specific for the (*a*) 5'Dδ1, (*b*) Jδ1, and (*d*) Jβ1 regions. (*c*) HindIII digests of BALB/c liver and KKF genomic DNA were hybridized to a probe specific for a region 5' of Dβ1. Germline bands are evident in the first lanes, and KKF rearrangements in the second lanes of each figure.

A probe specific for the Jδ1 region revealed two δ locus rearrangements, represented by 5.8- and 4.7-kb bands (Fig. 3 *b*). The 5.8-kb band also hybridized to a probe specific for the region just 5' of Dδ1 (Fig. 3 *a*), suggesting that it represented a partial (DDJ or DJ) rearrangement. The 4.7-kb band did not hybridize to this probe, indicating that it represented a rearrangement to regions upstream of Dδ. To rule out the possibility of aneuploidy, KKF was karyotyped; there were only two copies of chromosome 14, on which the α/δ loci reside (37). The presence of two δ rearrangements therefore demonstrated that the α genes on both chromosomes could not be rearranged by conventional deletion mechanisms.

KKF Does Not Express Surface TCR-δ. The possibility that KKF, like the human leukemia cell line DND41 (10), expressed a TCR-β/δ heterodimer was ruled out by the following observations. A single δ message is transcribed by KKF (Fig. 2). It is shorter than that reported (38) for a complete rearrangement (2.0 kb), and a sequence of this transcript (Fig. 4) showed that it represented the partial DDJ rearrangement predicted by Southern analysis; the 5' region

....CACTGTG GTGGCA TCCTTAT ATCGGAGGGAT ACGAGCT
7mer D1 D2

CGCACCGACAAACTCGTCTTTGGACAAGGAACCCAAGTGACTGTGGAACCA
J1

AAAAGCCAGCCTCCG....
C

Figure 4. Sequence of the δ transcript. KKF mRNA was subjected to anchoring PCR amplification and sequenced, revealing a partial Dδ1/Dδ2/Jδ1 rearrangement. The upstream region was in germline configuration as indicated by the presence of the heptamer sequence.

was in germline formation. As expected, KKF did not bind antibodies specific for TCR-δ proteins (Fig. 5). The rearrangement on the other chromosome involves regions upstream of Dδ. Because there is no transcription product that corresponds by size to a complete VDJ join, this rearrangement may involve the murine homologue of the δ-Rec sequence, which has been reported to play a role in progressive deletions of the δ locus before complete α rearrangement (39).

KKF Expresses a Fully Rearranged Vβ13 Gene. These data, taken together, indicated that KKF expressed surface TCR-β protein in the absence of TCR-α, -γ, and -δ chains. While the Jβ2 locus is unrearranged (data not shown), two rearrangements were apparent from hybridization of a probe to the Jβ1 region (Fig. 3 *d*). Hybridization of a probe to the region 5′ of Dβ1 (Fig. 3 *c*) indicated that one of these (6.8 kb) was a partial DJ rearrangement. The other chromosome was not represented, indicating that this region had been deleted due to a complete VDJ rearrangement. Probes hybridizing to specific Vβ genes were used in Southern and Northern blot analyses to determine which of the 20 odd Vβ regions was expressed. The Vβ13 gene was found to be rearranged and expressed at the RNA level (data not shown). That it was also expressed as a surface protein was demonstrated by the binding of MR 12-4, an antibody specific for the Vβ13 protein, to the surface of KKF (Fig. 1 *c*).

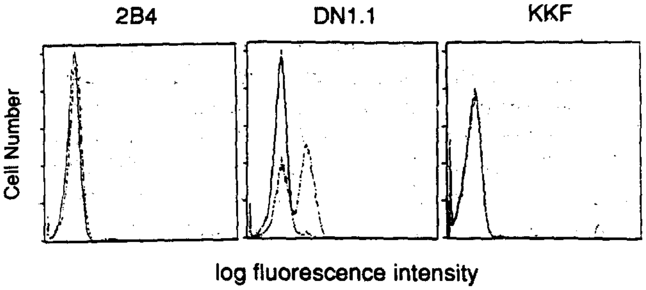

Figure 5. Surface expression of TCR-δ. One-color flow cytometric analysis of cells stained with antibody specific for Cδ (3A10). Staining of (*a*) 2B4 (TCR-α/β$^+$), (*b*) DN1.1 (TCR-γ/δ$^+$), and (*c*) KKF cells is indicated by the solid lines, negative controls by the dotted lines.

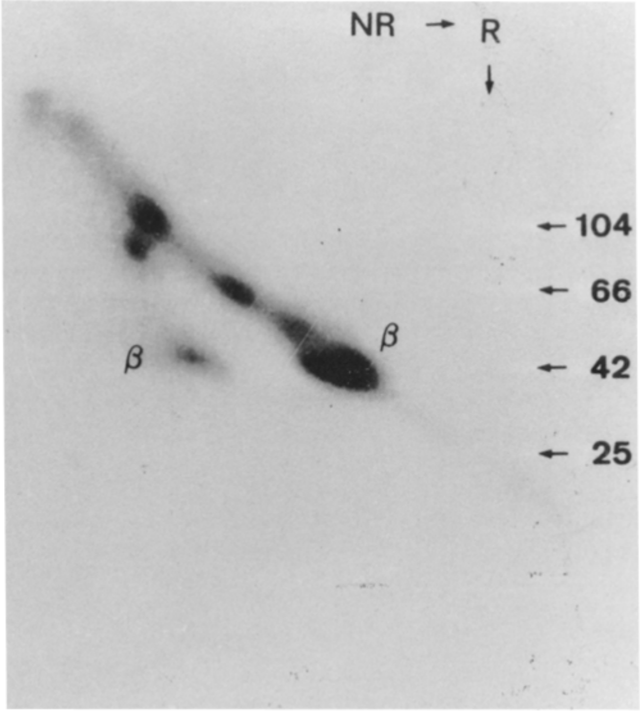

Figure 6. Surface expression of a TCR-β homodimer and monomer. ^{125}I surface-labeled KKF cells were lysed in digitonin and precipitated with H57-597 (anti-TCR-β). Precipitates were subjected to electrophoresis in two dimensions under nonreducing (NR) conditions and, subsequently, reducing (R) conditions. The disulfide-linked β homodimer is visible as a 42-kD spot off the diagonal, and the unlinked protein at the same relative molecular mass on the diagonal. Unlabeled spots represent nonspecifically precipitated proteins (confirmed when isotype-matched irrelevant antibodies were used to precipitate the lysate [data not shown]).

The TCR-β Chain Can Be Expressed on the Surface of KKF as Part of a Disulfide- and Nondisulfide-linked Complex. To determine the structure of the incomplete complex expressed by KKF, ^{125}I surface-labeled proteins were precipitated with antibodies specific for the constant region of TCR-β (H57-597). Two-dimensional electrophoresis of this precipitate revealed two proteins that correspond by size (42 kd) to the β chain (Fig. 6). One is found alone below the diagonal, suggesting that it is part of a disulfide-linked homodimer. The other 42-kD protein runs on the diagonal, suggesting that it is not involved in any disulfide linkage. A single 42-kD band is also evident after one-dimensional electrophoresis of H57-597 precipitates run under nonreducing conditions (data not shown).

Surface TCR-β and CD3-ϵ Are Weakly Associated. Immunoprecipitation results also suggest that the associations among the TCR-β chain and the CD3 complex are unique. Our data indicate that the interactions among the chains are significantly weaker than those observed in mature T cell clones and hybridomas. Fig. 7, *a–d* compares precipitates generated from lysates of KKF and KgV, a Gross virus–transformed T cell that expresses a conventional TCR-α/β heterodimer. A number of anomalies are apparent. First, CD3-ϵ and the unlinked TCR-β chains expressed by KKF do not coprecipitate under the mild, nonionic lysis conditions (1% digitonin), which have been shown to preserve the noncovalent associations among the complex proteins expressed by T cells studied thus far (40). While CD3-δ, -γ, and -ϵ proteins can be precipitated when anti-TCR-β is used with surface-labeled KgV lysates (Fig. 7 *a*), only CD3-δ and -γ are detected when precipi-

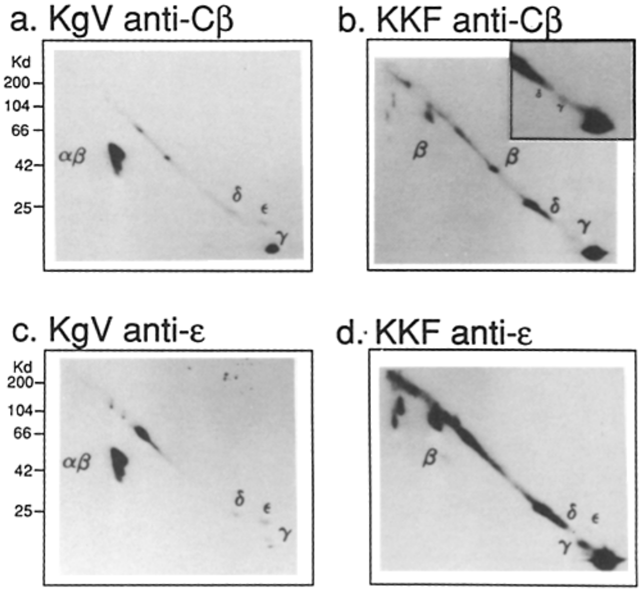

Figure 7. Immunoprecipitation of TCR/CD3 molecules expressed by KKF and by KgV: a comparison of the structures coprecipitated with anti-TCR-β and anti-CD3-ε. Digitonin lysates of surface-labeled KKF and KgV were precipitated with H57-597 (*a* and *b*) and 135 (*c* and *d*) and resolved by two-dimensional (2-D) electrophoresis under nonreducing (NR) and reducing (R) conditions. The TCR/CD3 components are identified. The disulfide-linked TCR-β proteins run at a higher molecular mass than the unlinked TCR-β, a possible consequence of differential glycosylation. The inset shown in *b* represents a prolonged exposure of the CD3 components precipitated by anti-TCR-β and resolved by 2-D electrophoresis. Unlabeled spots represent nonspecifically precipitated proteins.

tations are performed with KKF lysates (Fig. 7 *b*). The inset shows a prolonged exposure of the CD3 complex components precipitated by anti-TCR-β; again, CD3-ε is absent. It is, however, expressed on the surface of KKF; antibodies to CD3-ε coprecipitate surface CD3-δ, -γ, and -ε (Fig. 7 *d*). While the disulfide-linked ββ homodimer is evident after CD3-ε precipitation, little if any of the unlinked β proteins coprecipitate. Taken together, these data indicate that the TCR-β/CD3-ε complex expressed by KKF is more easily dissociated than has been observed in T cell lines to date and that the disulfide-linked β homodimer associates more effectively with CD3-ε.

The CD3-ζ Chain Is Weakly Associated with TCR-β or CD3-ε. KKF expresses CD3-ζ on its surface, as demonstrated by two-dimensional electrophoresis of surface-labeled cell lysates after precipitation with anti-ζ antiserum (Fig. 8 *a*). We have further evaluated the associations of the ζ chain with TCR-β and CD3-ε proteins with immunoblots of precipitates of whole cell lysates (Fig. 8, *b* and *c*). In the first experiment (Fig. 8 *b*), KKF and KgV were lysed with digitonin, immunoprecipitated with anti-TCR-β or anti-CD3-ε, and subjected to electrophoresis under nonreducing conditions. The proteins were transferred to nitrocellulose and probed with a combination of anti-ζ and anti-TCR-α antibodies. Both anti-β and anti-CD3-ε coprecipitated the 32-kD ζ homodimer and the 90-kD α/β heterodimer expressed by the control cell line, KgV. In contrast, neither antibody coprecipitated the ζ chain from the KKF lysates. We have, however, been able to detect an association among the ζ, CD3-ε, and TCR-β chains expressed by KKF by using the more sensitive monoclonal anti-ζ reagent, H146-968 (Fig. 8 *c*). Here, whole KKF

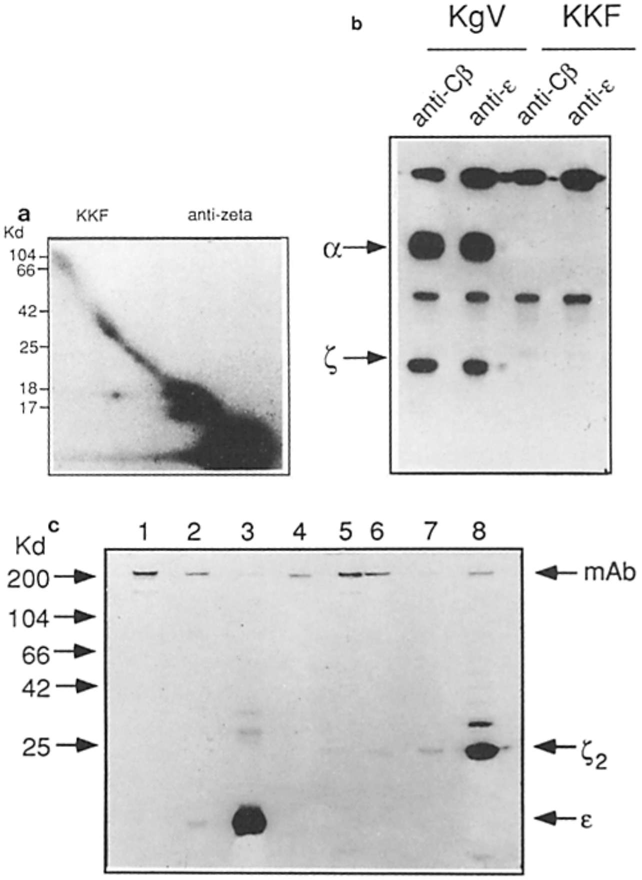

Figure 8. (*a*) Surface expression of CD3-ζ. Immunoprecipitation of ^{125}I surface-labeled KKF cells by anti-CD3-ζ antiserum. Precipitates were resolved by two-dimensional electrophoresis under nonreducing, then reducing conditions. The ζ chain is apparent as a 17-kD spot below the diagonal. (*b*) The relationship of CD3-ζ to TCR-β and CD3-ε: KgV vs. KKF. Whole cell lysates of KKF and KgV were precipitated with anti-Cβ (H57-597) or anti-ε (1352) and run under nonreducing conditions. Western blots of these precipitates were performed as described with a cocktail of antibodies specific for both TCR-α (the monoclonal, H28-760) and CD3-ζ (H146 antiserum). The bands corresponding to the α chain of the TCR heterodimer (90 kD) and the ζ homodimer (30 kD) are indicated. Little if any ζ chain is coprecipitated from KKF lysates with either anti-ε or anti-Cβ. As expected, TCR-α is not coprecipitated from KKF lysates. The bands with the highest molecular masses correspond to the antibodies used for immunoprecipitation, and the 67-kD bands present in each lane are nonspecifically precipitated. That the KKF lysate precipitations had been successful was shown by probing companion filters with antisera specific for TCR-β and CD3-δ (data not shown). (*c*) A detectable association among ζ, ε, and β chains. Whole cell lysates of KKF were precipitated with the mAbs H28-760 (anti-TCR-α) (lanes *1* and *5*), H57-597 (anti-TCR-β) (lanes *2* and *6*), 1352 (anti-CD3-ε) (lanes *3* and *7*), and H146-968 (anti-ζ) (lanes *4* and *8*). Western blots were performed as described with 1352 (lanes *1–4*) and H146-968 (lanes *5–8*). The positions of the ζ homodimer and CD3-ε are labeled. The content of the other bands present in lanes *3* and *8* is unknown.

cell lysates were precipitated with anti-TCR-α (lanes *1* and *5*), anti-TCR-β (lanes *2* and *6*), anti-CD3-ε (lanes *3* and 7), and anti-ζ (lanes *4* and *8*) mAbs. Lanes *1–4* were probed with anti-CD3-ε, and lanes *5–8* with anti-ζ A low level of the ζ homodimer is evident after precipitation with anti-TCR-β and anti-CD3-ε (lanes *6* and 7). The anti-ζ antibody does not coprecipitate detectable levels of CD3-ε (lane *4*), a possible consequence of the lower sensitivity of the anti-CD3-ε antibody when used as a blotting reagent.

These results indicate that the ζ chain associations with the TCR/CD3 complex are not as strong in KKF as they are in other cell lines studied. A recent report by Geisler et al. (9), demonstrating that an interaction between TCR-α and CD3-ζ chains is required for proper assembly and expression of the complete heptameric TCR/CD3 complex, is consistent with our finding that in the absence of an α chain, the ζ protein is weakly associated with the complex expressed by KKF.

KKF Responds Differently to TCR and CD3 Stimulation. Like normal DP thymocytes, DP subclones of KKF cannot be stimulated through their TCR/CD3 complex to release lymphokines (data not shown). We, therefore, examined the ability of KKF to mobilize Ca^{2+} after crosslinking of both TCR-β and CD3-ε surface proteins (Fig. 9). While the anti-TCR-β antibody, H57-597, stimulates mature peripheral T cells to release intracellular Ca^{2+} with the same kinetics as does the anti-CD3-ε antibody, 2C11 (19), these antibodies do not act equivalently on KKF. Anti-CD3 stimulation resulted in a rapid increase in intracellular Ca^{2+} (Fig. 9 *a*), in which the majority of cells (~60% [data not shown]) participated, while the rise seen after TCR stimulation occurred more slowly and did not achieve the levels seen after CD3 stimulation (Fig. 9 *b*). Fewer cells participated in the response and the response per cell was reduced in comparison (data not shown). Similar differences in response to anti-TCR-β and anti-CD3-ε stimulation has also been observed among a group of normal DP thymocytes (20), raising the possibility that KKF, which shares their DP surface phenotype, is a transformed representative of this subpopulation of cells. The functional uncoupling observed may be a direct consequence of the structural anomalies described.

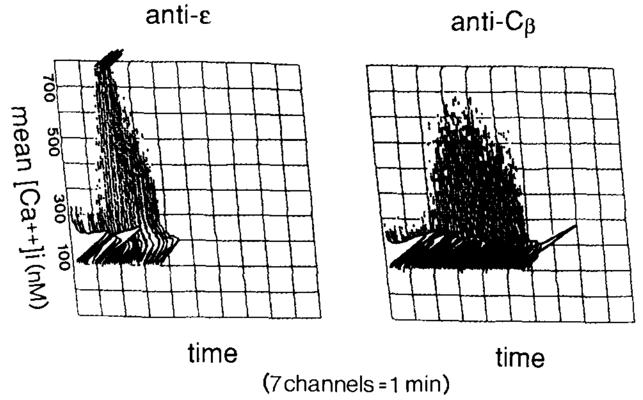

Figure 9. Intracellular Ca^{2+} release after stimulation of KKF with anti-CD3-ε and anti-TCR-β antibodies. Cells loaded with Indo-1 were stimulated with the antibodies 2C11 (anti-ε) and H57-597 (anti-C-β). Flow cytometric analyses were performed with cells immediately after addition of the secondary crosslinking goat anti-mouse antibody. The data are presented as concentration of mean internal Ca^{2+} (0–700 nM) on the *y*-axis vs. time (seven channels = 1 min) on the *x*-axis.

Discussion

We show that the transformed DP thymocyte, KKF, expresses disulfide-linked and unlinked TCR-β chains in the absences of TCR-α, -γ, and -δ proteins. The unlinked β chain, apparent as a 42-kD spot on the diagonal (Figs. 6 and 7) could exist on the surface of KKF as a β "monomer", but is as likely to be present as an unlinked homodimer or multimer. These findings have both structural and developmental implications. First, they challenge what is currently understood about TCR/CD3 assembly. Studies show that TCR/CD3 components are degraded or retained intracellularly if they do not become part of a complete multimeric (TCR-α/β/CD3-γ/δ/ε and, usually, TCR-ζ-ζ or -ζ-η) complex (2–6, 8, 41). While it has been assumed that the TCR heterodimer was a necessary part of this complex, our data and that of Goverman et al. (11) demonstrate clearly that a disulfide-linked β-β dimer can substitute. Our data further suggest that noncovalently linked TCR-β proteins can reach the surface successfully.

A number of T cell lines that express functional TCR-β but not -α proteins have been examined and no evidence for surface expression of the CD3 complex or the β chain has been found. There are several possible explanations for the differences in our observations. First, the KKF β chain may be unique, perhaps in the transmembrane region that recently has been defined as the site that targets an uncomplexed TCR-α protein for intracellular degradation (6). However, we have recently sequenced the KKF β chain and no unusual DNA sequences have been found in this region. Second, it is possible that only certain V-β proteins can be expressed in the absence of TCR-α. Finally, KKF may produce another protein(s) that permits expression of incomplete complexes by assisting assembly and/or inhibiting degradation. Given KKF's thymocyte origins and phenotype, it is possible that expression of this protein is developmentally regulated.

It is tempting to speculate that TCR-β homodimer expression is a feature of a specific stage during T cell development. Because the β locus is rearranged and expressed as a cytoplasmic protein before TCR-α expression (42), early thymocytes necessarily pass through a stage at which the β chain has the opportunity to be expressed in the absence of TCR-α. The recent finding by Kishi et al. (12) that a transgenic TCR-β chain can be expressed on the surface of normal DN and DP thymocytes in the absence of other TCR proteins supports this possibility. Unlike KKF, these thymocytes do not seem to express surface CD3. However, because of the heterogeneity of the populations examined, it remains possible that a small percentage of cells do coexpress TCR-β and CD3, a phenotype that may represent the next step in development, occurring before TCR-α expression. That there have not been more reports of such a complex in vivo may be due in part to the difficulty of assessing the independent expression of TCR-α and -β chains within a heterogeneous population of cells with the reagents available to date.

Provocatively, the dominant phenotype of KKF cells is that of developing DP thymocytes (J11d$^+$/CD4$^+$/CD8$^+$/CD3$^+$), which have been shown by Finkel et al. (19, 20) to be heterogeneous in their response to TCR/CD3 stimulation. A subpopulation of TCR$^+$ DP thymocytes is responsive to stimulation through both TCR-β and CD3-ϵ, while another is responsive only through CD3-ϵ. Our finding of a functional uncoupling among the TCR chains and CD3 complex in this transformed DP thymocyte line parallels this group's observation. Furthermore, the weak association between the TCR and CD3 proteins, suggested by our observations of KKF, could account for the functional anomalies that define this subgroup of normal DP thymocytes. The weak association between the TCR/CD3 complex and the ζ homodimer may be of particular significance given the role that the ζ chain plays in T cell activation (43).

It has been proposed that the subgroup of DP thymocytes exhibiting a functional uncoupling between the TCR β chain and the CD3 complex are targets for positive selection (20), a process responsible for establishing MHC restriction specificity. Given the implications of our data, it is possible that this subgroup expresses the TCR-β chain in the absence of TCR-α. Evidence that certain V-β proteins preferentially recognize specific MHC molecules (reviewed in reference 16) raises the possibility that the β chain alone may be responsible, in some cases, for the restriction specificity of a T cell. While other studies demonstrate that the α chain has an influence on the positive selection of cells expressing TCR-β transgenes (44–47), it is possible that a thymocyte expressing a β chain homodimer (structurally and functionally unlinked to CD3 transduction machinery) could be positively selected according to the ability of the β chain to bind specific MHC molecules. This thymocyte could subsequently gain expression of an α gene, become functionally coupled to the CD3 transduction machinery and finally be screened for self-reactivity (negative selection). This scenario could help explain how thymocytes seem to be positively selected on the basis of a broader range of TCR specificities and affinities (self-restriction) but negatively selected on the basis of high affinity to very specific ligands (self-tolerance).

Further studies of KKF and other DP cell lines may lead to a better understanding of both the requirements for expression of the TCR structure and the dynamic role TCR/CD3 structure and the function play in T cell development.

We are grateful to Dr. Susan Faas for helpful discussions and for critical readings of the manuscript. We also would like to thank Drs. Itohara and Tonegawa for the anti-Cδ antibody, 3A10, Dr. Osami Kanagawa for antibodies to Vβ13 and Vβ9, Dr. Dennis Loh for probes to the V-β regions, Dr. Alan Korman for the 5'Dδ probe, and Dr. Donna George for karyotyping KKF.

Y. Hashimoto, J. Punt, and S. Kathiresan were supported by the University of Pennsylvania Research Foundation, the American Cancer Society (IM-576), and, in part, by the National Institutes of Health (AR-39910), Y. Hashimoto is a special fellow of the Leukemia Society of America, Inc., and J. Punt is a Veterinary Medical Scientist Training Program (VMSTP) trainee. R.T. Kubo is supported by NIH grants AI-18785, AI-17134, AI-26880, and P01 AI-22295. T. Saito is supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture and the Life Science Research Project of the Institute of Physical and Chemical Research (RIKEN). T. H. Finkel is a recipient of an Arthritis Foundation Post-doctoral Fellowship.

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Received for publication 4 March 1991 and in revised form 10 June 1991.

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